# *Microbiology of Human Spacecraft Environments*

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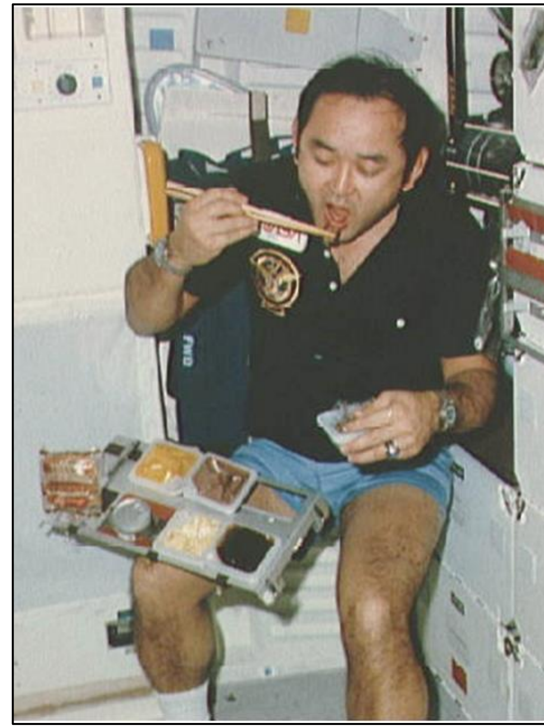
# Microbiology and Space Missions

- Astronaut health
- Spaceflight foods
- Vehicle habitat
  - Life support systems
  - Trash and human waste containment
  - Astronaut hygiene areas
- Planetary protection
  - How do we protect the crew and extraterrestrial environments in our search for life on other planets?



**These priority areas for microbiology drive vehicle design, microbial monitoring, and operational research efforts**

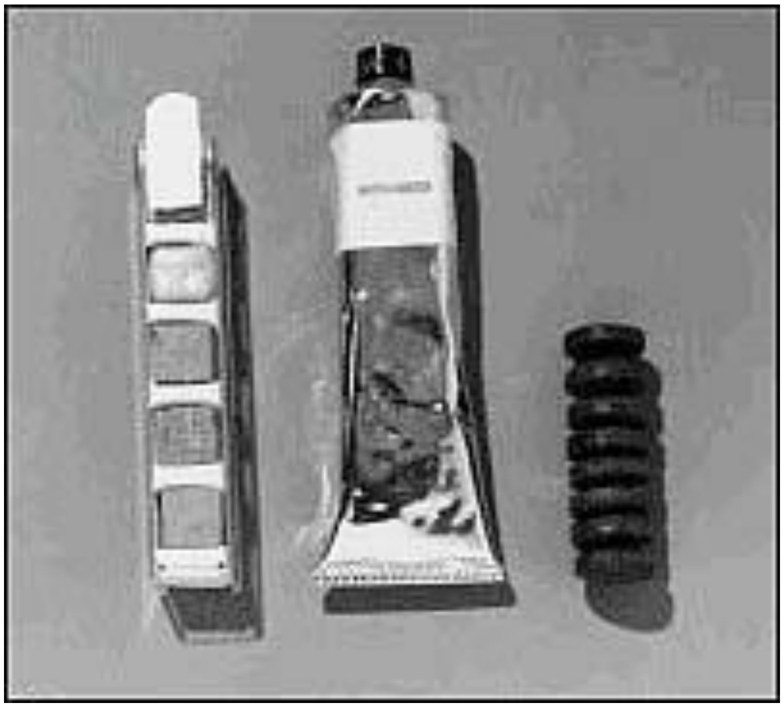
# Astronaut Health

- Diagnosis is often based on symptomology (*e.g.*, headache, rash, dry hacking cough, diarrhea)
- Examples of infectious diseases during spaceflight missions include upper respiratory infections, urinary tract infections, ear infections, herpes zoster, rashes and skin disorders, and gastroenteritis.
- Survey of Space Shuttle missions (STS-1 through STS-89) indicated infectious disease accounted for 1.4% of all medical events (not including skin and subcutaneous tissue)
- Evidence of immune system dysfunction

**Why do exceptionally healthy astronauts in a pristine environment still contract infectious diseases?**

Risin, D. in *Human Health and Performance Risks of Space Exploration Missions,* (NASA SP-2009-3405, 2009).

# Spaceflight Food

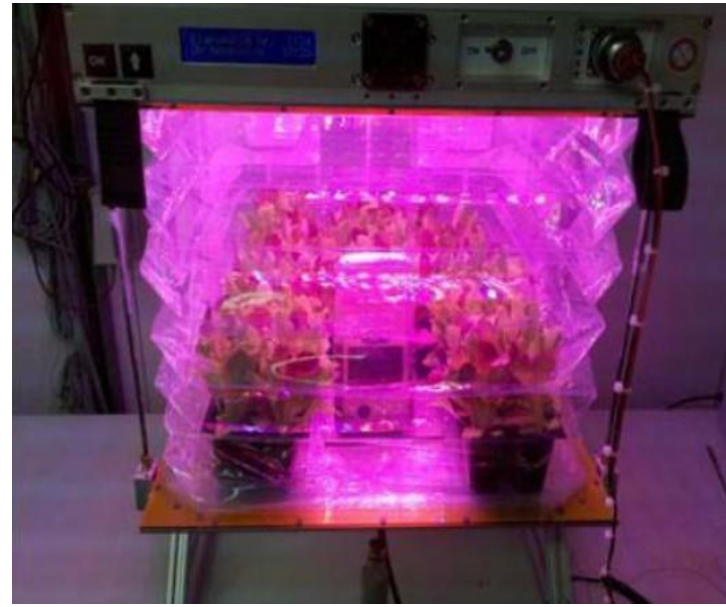


# Spaceflight Food

- Several categories
  - Fresh foods
  - Irradiated foods
  - Thermostabilized foods
  - Non-thermostabilized foods
- Non-thermostabilized foods
  - Includes dehydrated foods, tea, food bars, and cheese
  - Samples from each production lot are tested
  - Lots have been disqualified after detection of microbes, including the bacteria *Salmonella enterica* Typhimurium, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and fungi *Aspergillus fumigatus*, and *Aspergillus flavus*.

# The Future of Spaceflight Food

- The VEGGIE spaceflight experiment grew lettuce on the International Space Station (ISS)
  - Can the crew eat it?
- Probiotics are being evaluated as an immune boosters
  - What organisms do we monitor for safety?
- We are planning to prepare and cook more food during flight
  - How do we control contamination during processing?



# Vehicle Habitat - Spacecraft Design

- Vehicle surfaces
  - Use of materials that are not conducive to microbial growth
- Vehicle air
  - Use of High Efficiency Particulate Air (HEPA) filters
- Potable water recovery system
  - Potable water can be brought on a mission, or recycling humidity condensate and/or urine may be more efficient
  - On ISS, recycled water experiences a catalytic oxidizer (267°F for 10 minutes); iodine disinfection; and passes through a 0.2-micron filtration

# Vehicle Habitat - Operations

- Health Stabilization Program
  - Preflight quarantine to decrease the risk of infectious disease
  - Employs other prevention techniques, such as raising infectious disease awareness
- Preflight disinfection and microbial monitoring
- Inflight cleaning and remediation
  - Regular housekeeping
  - Disinfectant wipes for contaminated surfaces
  - High concentration iodine solution to disinfect potable water systems

# Contamination Potential

***Spacecraft are complex***

***Trash and waste control***

***Astronaut activities, such as eating and hygiene***

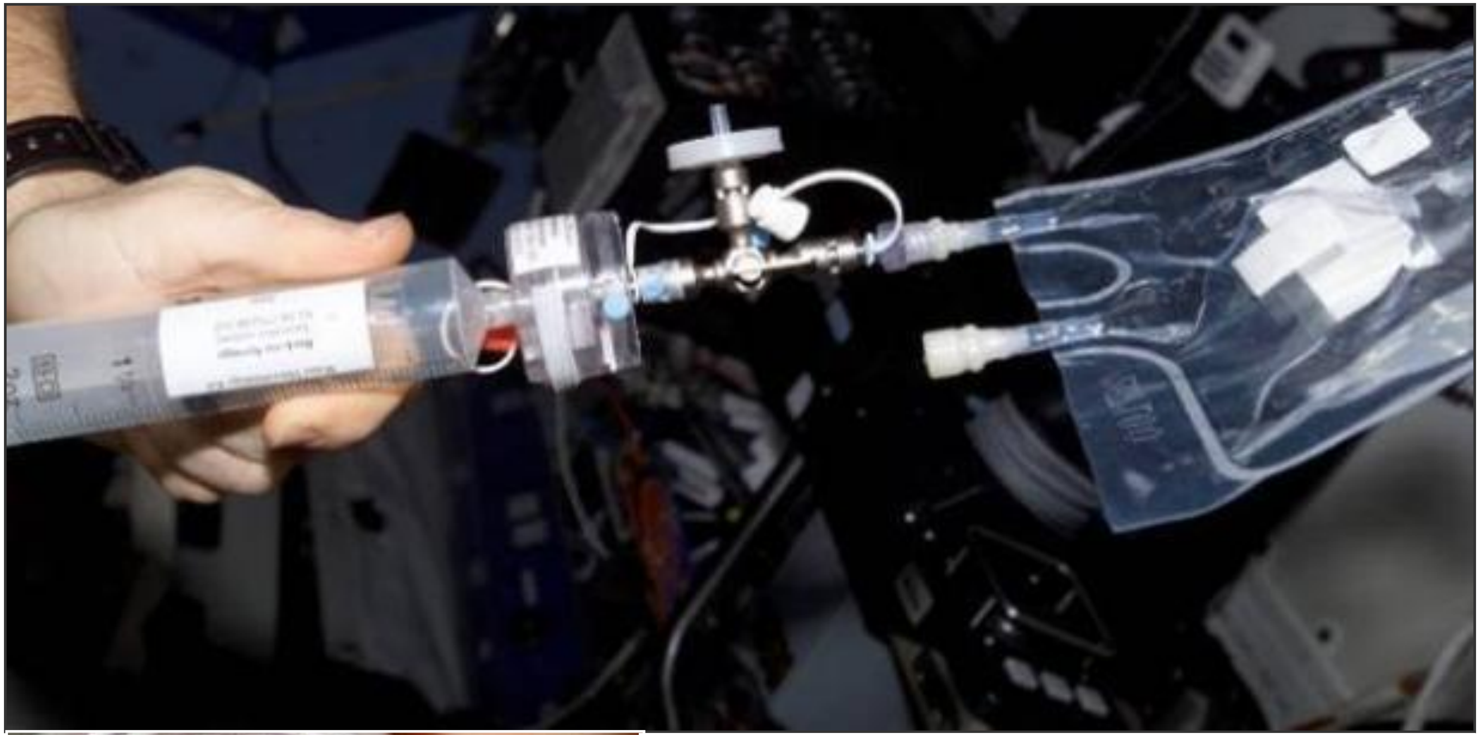
# Microbial Monitoring on the ISS

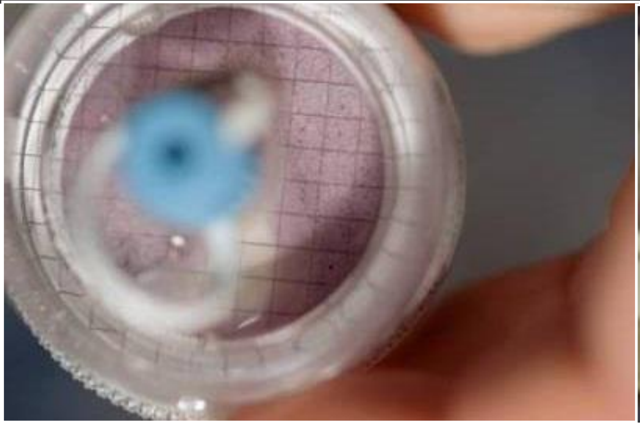
**Surfaces** | **Air** | **Water**


ISS020E017956

Quantified in-flight and returned to JSC for identification

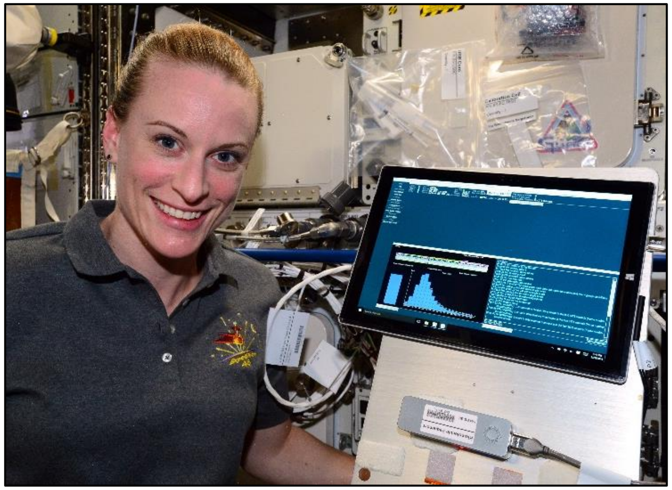
# Microbial Monitoring of Water





# Advances in Microbial Monitoring

- DNA sequencing in space
  - First performed in 2016 using Oxford Nanopore's MinION technology
- In-flight identification of environmental samples
  - Three microbial colonies from a media plate used for ISS surface sampling were processed and sequenced on ISS.
  - The MinION accurately identified the three isolates that were selected.
- Following up with Swab to Sequencer direct analysis



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Stahl-Rommel, S., *et al.* *Genes* **12**, 106 (2021).

# Environmental Health

**Microbial monitoring by NASA and others indicate ISS environmental flora reflect human-associated microorganisms commonly found in terrestrial homes.**


- Media-based monitoring occasionally identifies opportunistic bacterial pathogens, such as *Staphylococcus aureus* and *Bacillus cereus*. No methicillin resistant *S. aureus* (MRSA) has been identified.
- *Enterobacter* and *Enterococcus* species are occasionally identified throughout the ISS.

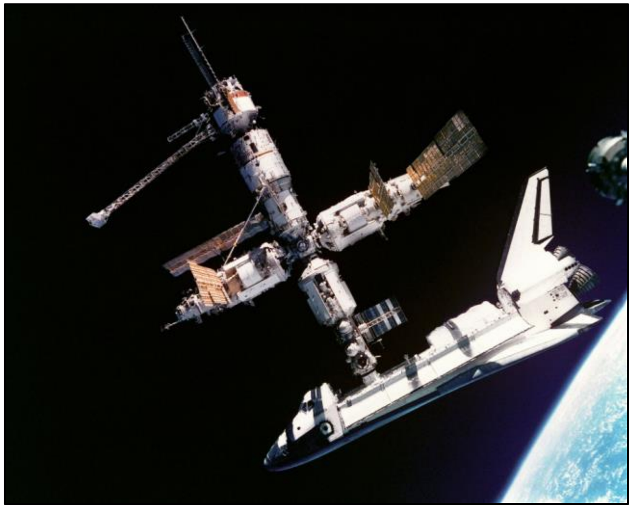
Pierson, D., *et al.* in *Environmental Monitoring: A Comprehensive Handbook* (DHI Publishing, 2012)
Blaustein, R. A., *et al. mSystems* **4**, doi:10.1128/mSystems.00281-18 (2019)

# Environmental Health

- The most prevalent fungal genera are ***Aspergillus*** and ***Penicillium***. ***A. flavus***, ***A. niger***, and ***A. fumigatus*** have been identified from spaceflight samples. ***Stachybotrys chartarum*** has been isolated preflight.
- Samples from potable water (for drinking and hygiene) indicate common water-borne bacterial species.


**As ISS has many visiting vehicles with astronauts, "new" bacterial and fungal isolates are regularly identified.**

# Environmental Anomalies



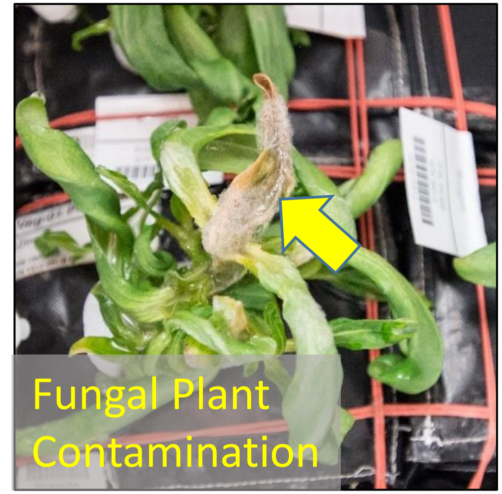
- Russian Mir Space Station (1986-2001)
  - Samples of free-floating condensate caused by power failures and problems with temperature control revealed high microbial diversity, including ***Escherichia coli***, ***Serratia marcescens***, ***Legionella* species**, and **protozoa**
- International Space Station (1998-present)
  - Surface contamination is often associated with uncontrolled water
  - Air contamination and unpleasant odors do occur; however, the sources are often unknown. These events have been transient and/or handled by the HEPA filtration system.
  - Biofilm formation in ISS water lines have caused clogged lines and pump failure.

Ott, C. M., *et al.* *Microb Ecol* **47**, 133-136 (2004)

# ISS Environmental Anomalies

Fungal Contamination in ISS Module

Bacterial Biofilm in ISS Drinking Water System



Fungal Plant Contamination

Fungal Contamination in ISS Cabin Air

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# Microbiological Research in Space

- Multiple spaceflight experiments over the past 50 to 60 years have demonstrated unexpected microbial responses when microorganisms are cultured during spaceflight, including:
  - Antibiotic resistance
  - Biofilm formation
  - Growth rates
  - Virulence
  - Gene expression
- Rotating Wall Vessel (RWV) bioreactor
  - Fluid movement in the reactor simulates several aspects of the microgravity environment
  - Provides predictive and follow-up studies for spaceflight experiments

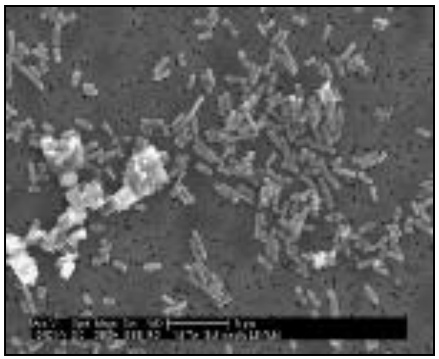
The Fluid Processing Apparatus (FPA), an example of in-flight hardware

The low fluid shear culture conditions has prompted the term Low Shear Modeled Microgravity (LSMMG) environment

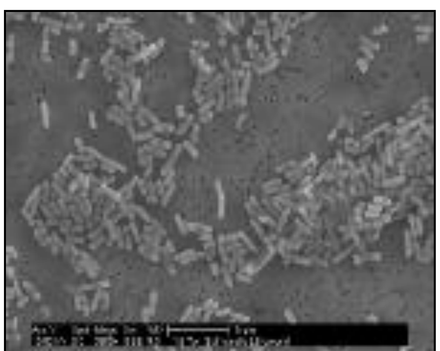
# MICROBE: *Salmonella* Response to Spaceflight Culture

**PI: Dr. Cheryl Nickerson, Arizona State University**

Flight Sample



Ground Control



- In 2006, the MICROBE experiment compared the characteristics of *Salmonella* Typhimurium grown in space to otherwise identical cultures grown on the Earth.
  - First to identify alterations in microbial virulence in a spaceflight grown microorganism
  - First transcriptomic study of a microorganism grown in space
  - First identification of a spaceflight molecular response mechanism
  - First to identify novel changes in biofilm production

Wilson, J. W. *et al. Proc Natl Acad Sci U S A* **104** (2007)

# Astronaut Microbiome

**PI: Dr. Hernan Lorenzi, J. Craig Venter Institute**

- First comprehensive study of the microbiomes of nine astronauts on ISS
- Samples included skin sites, nostrils, fecal samples collected preflight, in-flight, and post-flight
- Composition of the intestinal microbiota became more similar across astronauts in space
- Alterations in the skin microbiome that might contribute to the high frequency of skin rashes
- Set the baseline for astronaut microbiome studies with low sample size

Voorhies, A. A. *et al. Sci Rep* **9** (2019)

# Selected Current Studies

**PI: Dr. Mark Ott, NASA**
**Co-PI: Dr. Cheryl Nickerson, Arizona State University**

- Determine if growth in the **spaceflight analogue** **(RWV)** environment increases virulence in other bacteria, five pathogenic species are being investigated to characterize changes in stress response, adhesion/invasion of 3-D tissue culture models, and animal testing if warranted.

**PI: Dr. Cheryl Nickerson, Arizona State University**

- Investigate the combined effect of **spaceflight analogue** **(RWV)** culture with low dose radiation to determine if the combination synergistically increases the potential infection risk.

**PI: Dr. Bob McLean, Texas State University**
**Co-PI: Dr. Cheryl Nickerson, Arizona State University**

- Investigate polymicrobial biofilm development by *Pseudomonas aeruginosa* and *Escherichia coli* during **spaceflight** to identify changes in biofilm architecture, disinfection, and corrosion potential during spaceflight.


QUESTIONS???